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Patent and Trademark Office
US Department of Commerce
Appn. Number: 10/007,489
Appn. Filed: 12/05/2001
Applicant: Elizabeth Gay Frayne

Title: "Microbial Production of Phosphorothioate Substituted DNA, RNA, and Oligo Mixtures"

Examiner: Devesh Khare, PhD, JD
Art Unit:1623

RE: Office Action Summary issued Nov. 28th for Application No. 10/007,489

Dear Sir,

I have modified my claims which I hope will be more acceptable. My invention deals with the in vivo incorporation of thio-phosphate into nucleotide precursor pools and ultimately into nucleic acids resulting in modified internucleotide linkages. Inorganic phosphate has two points of metabolic entry, namely during glycolysis and/or during oxidative respiration. From either of these points the modified phosphate is then incorporated into ATP and ultimately into nucleotide precursors and nucleic acids. This is the truly novel aspect of the invention which is not contested by your response. Therefore, I have tried to make it more clear in my claims that I mean in vivo and not in vitro. Sayers work does indicate that nucleotide triphosphates modified with an alpha thio-phosphate can be ultilized by DNA polymerase in vitro. However, my work shows that by simply culturing cells with thio-phosphate instead of inorganic phosphate the modified phosphate can be incorporated into ATP etc. Sayers work in no way addresses this issue as the synthesis of phosphorothioate DNA is done entirely in vitro using nucleotide precursors.

Concerning a method for generating oligos my work does show that phosphorothioate polymers can be digested with S1 nuclease and DNase I despite previous indications otherwise. The key to getting DNase to work was the use of a buffer optimized for single-stranded DNA digestion or one that containes Mn instead of Mg. S1 was found to be stereo-selective on short polymers. However, when I tested it on longer polymers generated in vivo (Rp configuration) S1 was able to digest the modified polymer. Digestion with these enzymes is important for

generating the greatest diversity of oligos spanning the insert. That is an overlapping set of oligos owing to the random nature of cleavage by these enzymes.

As regards the actual claims, claim one was totally reconstructed. This created the need for an additional claim or claim six containing bits of the original claim one. Other claims were modified a bit as indicated.

Respectfully submitted,

Elizabeth Frayne

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